Supplement to the manuscript by

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Entitled:

Low molecular weight DNA replication intermediates in *Escherichia coli*: mechanism of formation and strand specificity

Supplemental Figures

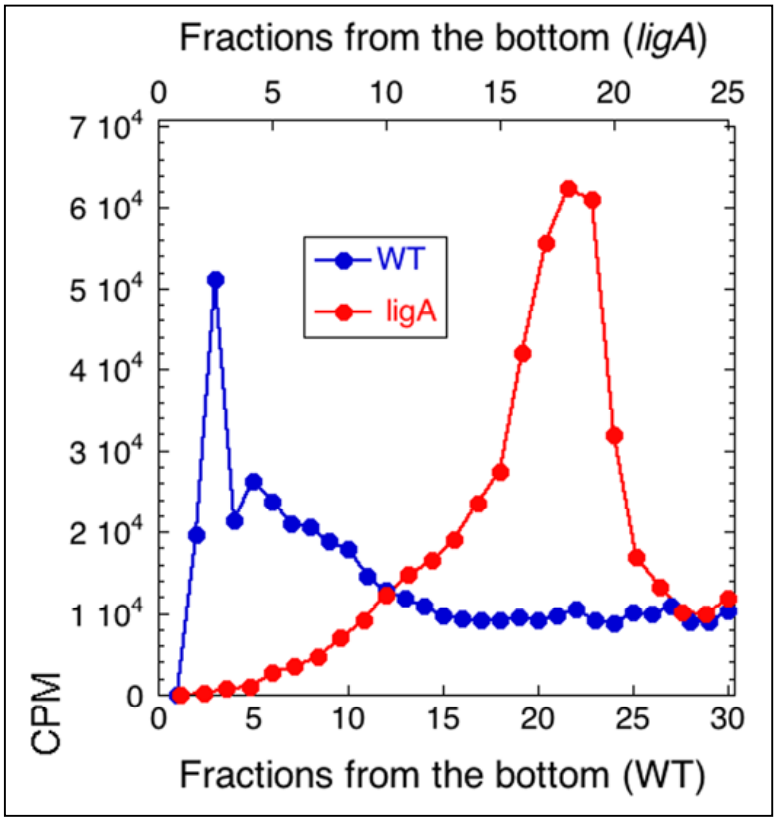

Fig. S1. When cells are lysed directly on the gradient, the profile of the replication intermediates from the *ligA* mutant cells does not change.

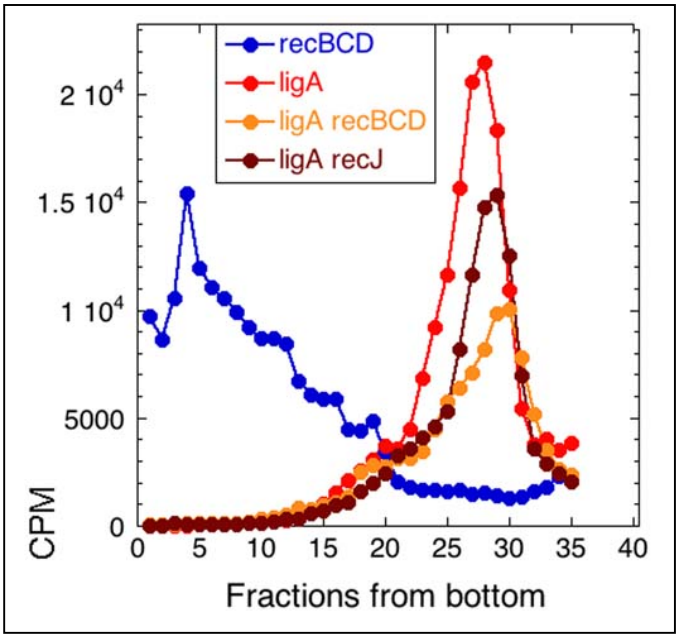

Fig. S2. Linear duplex DNA processing by RecBCD and ssDNA degradation by RecJ do not contribute or alter the profile of the replication intermediates in the *ligA251* mutant.

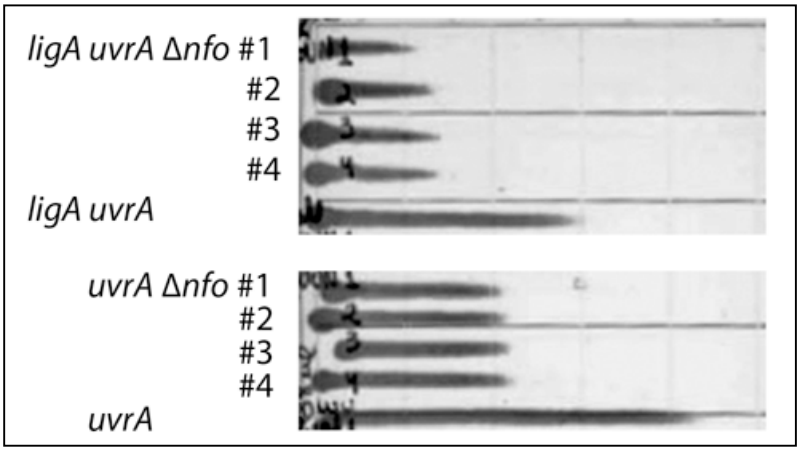

Fig. S3. Phenotypic test for the *nfo* defect: increased sensitivity to *tert*-butyl hydroperoxide, revealed on a gradient plate. The ligase mutant derivatives also show an additional increased sensitivity to t-BHP compared to LigA+ strains.

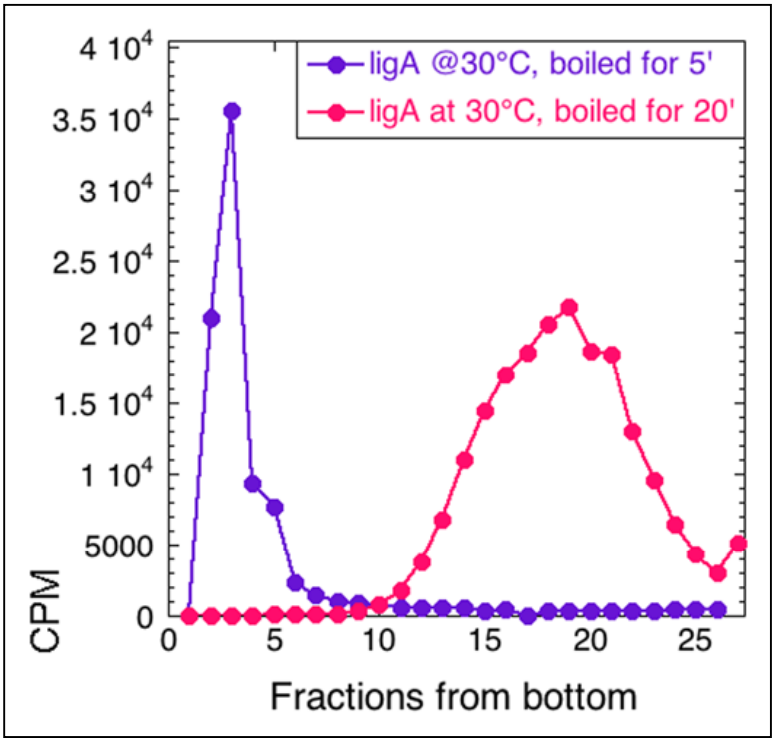

Fig. S4. Boiling not only denatures DNA strands, but also breaks them. The *ligA251* mutant was pulse-labeled at 30°C, when all the intermediates are maturated normally, and the DNA was run in the neutral sucrose gradient after boiling for 5 and 20 minutes. The expected HMW DNA strands are all fragmented by 20' boiling.

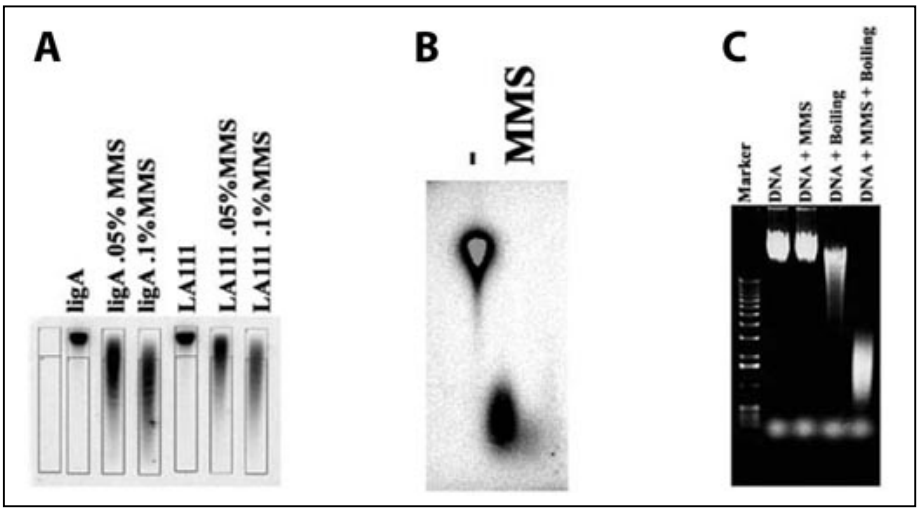

Fig. S5. Alkylation makes DNA sensitive to high temperatures and high pH.

A. ^{32}P-labeled stationary cultures of GR501 (*ligA251*) and of its excisionless variant (LA111) were treated with the indicated concentrations of MMS for 30 minutes at 42°C, the total DNA was isolated by phenol extraction and ran on an alkaline agarose gel. The direct radioactivity scan shows quantification boxes along the lanes. Only small difference is usually detected between the two strains. Essentially the same results were obtained with MMS treatments at 30°C and 37°C and for 15 minutes.

B. Purified ^{32}P-labeled chromosomal DNA was treated in vitro with 0.05% MMS at 42°C for 30 minutes and without ethanol precipitation loaded on an alkaline agarose gel (together with an untreated control).

C. Purified genomic DNA was treated or not with 0.05% MMS in vitro at 42°C for 30 minutes before being boiled or not for 10 minutes and ran on a neutral agarose gel.

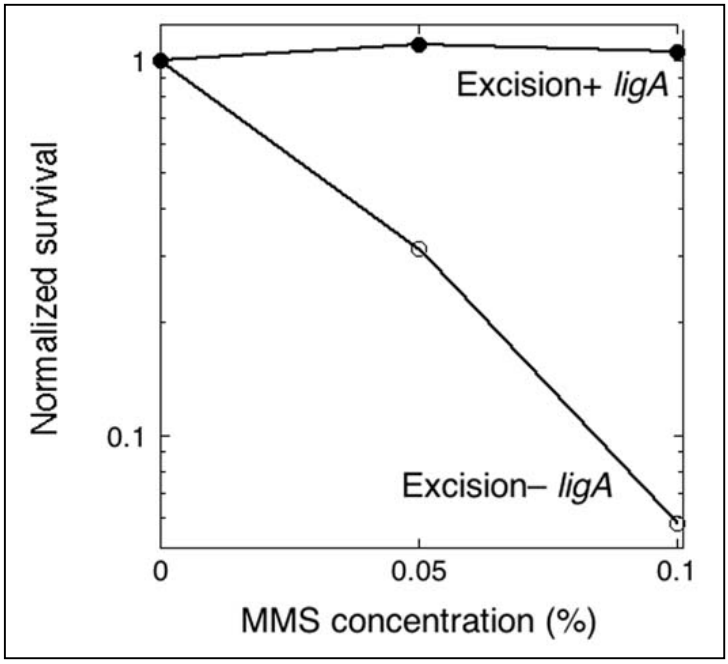

Fig. S6. Sensitivity of the excisionless strain to MMS.
Method: Cultures were grown in LB at 30°C until OD 0.3, at which point they were split into three parts, and to two of them MMS was added to the indicated concentrations. Cultures were incubated without shaking at 37°C for 15 minutes, and the surviving titer was determined after spotting serial dilutions on LB plate and incubation at 28°C. The strains are: Excision+ *ligA*, GR501; Excision– *ligA*, LA111.